MICROBIAL FLORA OF PACKAGED FRANKFURTERS AND THEIR RADIATION RESISTANCE[a]

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It has been reported previously from this laboratory *(13)* that the bacteria primarily responsible for the spoilage of refrigerated fresh beef are pseudomonads that are relatively sensitive to high energy radiations. When these organisms are killed by low doses of irradiation the refrigerated shelf-life of the meat is extended several-fold, but ultimately it spoils due to microorganisms that grow much more slowly at low temperatures than do the pseudomonads. Prominent among the more radiation-resistant spoilage microorganisms was a gram-positive organism which appeared to be identical to *Microbacterium thermosphactum,* as described by McLean and Sulzbacher *(7)*.

The present report concerns a similar study of the microbial flora of packaged frankfurters before and after irradiation. As a result of the bacteriostatic effect of curing ingredients, the bacteria present on frankfurters are predominantly gram-positive types. Ogilvy and Ayres *(9)* have reported that micrococci, bacilli, and sarcinae were most numerous, while lactobacilli and gram-negative bacteria were present in smaller numbers. Also, appreciable numbers of yeasts sometimes were encountered. They also studied the effect of increased CO_2 tension on the flora and found that as the CO_2 concentration was increased molds, yeasts, and micrococci were progressively inhibited and lactobacilli became predominant.

EXPERIMENTAL METHODS

Cellophane-wrapped frankfurters were purchased in local retail stores. Thirty-six packages from 11 different companies were examined. Duplicate samples were incubated at 2° and 10° C. until surface sliming became evident. Some of this slime was scraped off with a sterile scalpel, diluted in distilled water, and plated in APT agar *(4)*. The plates were incubated for 5 days at 25° C. Ten representative colonies were picked from the appropriate dilution of each sample, and bacteria were identified according to methods in the *Manual of Methods for Pure Culture Study of Bacteria (2)*. Yeasts were cultivated in MY broth *(5)* and identified according to the methods of Wickerham *(10–12)* and Lodder and Van Rij *(6)*.

The radiation resistance of pure cultures was determined as previously described *(13)*. A number of experiments were conducted to determine the effect of irradiation on the naturally-occurring microbial flora of frankfurters. Frankfurters from local retail stores were cut longitudinally and vertically to make 4 samples from the same frankfurter. Two of the quarters were individually wrapped in oxygen-permeable, moisture-proof cellophane and the other two were vacuum packaged in impermeable cellophane-polyethylene bags. After irradiation the samples were placed in a covered pyrex dish containing a beaker of water to supply a high humidity. These dishes were then held at 10° C. or at

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2° C. The bacterial counts were determined immediately after irradiation and after varying intervals of incubation. Colonies were picked and identified as previously described.

RESULTS AND DISCUSSION

Microbial flora of the slime on refrigerated frankfurters. A total of 353 microorganisms were isolated from 36 packages of frankfurters and were tentatively identified. Of these, 275 were bacteria and 78 were yeasts. The results are summarized in Table 1. Microbacteria and yeasts constituted the

TABLE 1

Microorganisms in the surface slime of refrigerated frankfurters

Isolates	From frankfurters stored at		Total number
	2° C.	10° C.	
Lactobacilli (heterofermentative)	14	28	42
Lactobacilli (homofermentative)	11	13	24
Leuconostocs	12	32	44
Microbacteria	61	41	102
Micrococci	11	29	40
Streptococci	4	8	12
Yeasts	48	30	78
Gram-negative rods	6	5	11
Total	167	186	353

two most frequently isolated groups of organisms and both of these were more common on franks stored at 2° C. than on those stored at 10° C. At 10° C., lactobacilli, leuconostocs, and micrococci were of greater relative significance.

Eighty-three representative bacteria were studied more extensively. Twenty-three strains of microbacteria were studied, and all proved to be very similar or identical to *Microbacterium thermosphactum (7, 13)*. The only differences noted were the production of acid from glycerol by 15 strains, the fermentation of lactose by 3 strains, and the fermentation of galactose by 6 strains.

Twenty-four strains of lactobacilli were studied (16 heterofermentative strains and 8 homofermentative strains). All of the lactobacilli grew at 2° C., and 12 of the heterofermentative and 6 of the homofermentative strains also grew at 45° C. All of the lactobacilli could produce hydrogen peroxide during growth, and all could cause greening of Vienna sausage. Most of the heterofermentative strains were identical with *Lactobacillus viridescens (8)*. The homofermentative strains did not coincide well with any generally recognized species *(1)*.

Fifteen *Leuconostoc* strains were studied. Eleven of them produced a polysaccharide slime from sucrose and 12 of them produced hydrogen peroxide. They all grew at 2° C. and 30° C., but only one grew at 45° C. Two strains were identified as *Leuconostoc mesenteroides* and the remaining 13 as *Leuconostoc dextranicum (1)*.

The 6 streptococci appeared to be enterococci, but differed from each other and from described species in a number of physiological tests. The 12 micrococci were extremely heterogeneous, but one proved to be a typical coagulase-positive *Staphylococcus aureus*. Three tetracocci were studied and were quite similar, but they were not identified specifically.

The gram-negative rods were not studied since they appeared to be of little significance, but they appeared to be pseudomonads.

The yeasts were studied quite extensively, and will be the subject of a separate report.

Radiation resistance of the microbial flora of frankfurters. The radiation resistance of 40 representative microorganisms is presented in Table 2. All of these were considerably more resistant than the pseudomonads from fresh meats, which had an LD_{99} of about 6500 rads under similar conditions. Most of these organisms from frankfurters were of the type that grow in chains or clumps and showed the multi-hit type of survival curve. The radiation resistance of some of these organisms was determined several different times, and the results agreed within approximately 11%.

TABLE 2

Radiation resistance of representative microorganisms from the surface of frankfurters

Organism	No. of strains	LD_{99}[1] rads x 10^{-3}
Lactobacilli (heterofermentative)	4	43–74
Lactobacilli (homofermentative)	3	172–209
Leuconostocs	5	40–113
Microbacteria	4	61–89
Micrococci	3	67–98
Staphylococcus aureus	1	44
Streptococci	3	81–236
Tetracocci	2	173–215
Yeasts	15	49–257

[1] Dosage for 99% destruction in TY broth (1.0% tryptone and 0.5% yeast extract).

Irradiation of frankfurters. The franks purchased in local grocery stores generally had an initial population of 10^4 to 10^6 microorganisms per gram. However, some packages exhibited very low counts. These franks were packaged in cellophane or were vacuum packaged, and irradiated as described under Experimental Methods. Seven experiments of this type were conducted, and the results with the cellophane-wrapped franks in four of these are summarized in Table 3. The results of the other three experiments were very similar, and the results with vacuum-packaged franks were not significantly different from those that were cellophane wrapped. However, the vacuum-packaged samples had a much better texture and color after prolonged storage even though conditions were quite humid.

It can be noted that an irradiation dosage of 140,000 rads reduced the initial microbial population about 99%. This appeared to delay microbial spoilage for 2 or 3 days at 10° C., and somewhat longer at 2° C. However, even this level of irradiation adversely affected the color of the product in both types of packages, especially the cut surface. These results are consistent with the results of Erdman and Watts *(3)* who reported that 186,000 rads adversely affected the color of bologna and was inadequate to extend the keeping time at room temperature.

Experiment No. 6 (Table 3) illustrates the importance of the initial population level. These franks had a very low microbial population before irradiation, and 230,000 rads killed virtually all of the organisms that could grow at 2° C. They showed no growth after 3 weeks, but after 5 weeks' incubation

TABLE 3

Effect of irradiation and storage on the microbial population of cellophane-packed frankfurters

Experiment No. and storage temp.	Irradiation (rads)	Bacterial count after			
		0 days	5 days	10 days	21 days
No. 2	0	7200	33×10^7	36×10^7	
	140,000	600	50×10^6	59×10^7	
10° C.	230,000	140	10×10^6	30×10^7	
	460,000	30	11×10^4	10×10^6	
No. 3	0	82×10^4	18×10^7	99×10^7	
	140,000	78×10^2	18×10^6	31×10^7	
10° C.	230,000	21×10^2	47×10^5	33×10^7	
No. 6	0	200	3300	11×10^5	27×10^7
2° C.	140,000	<10	<10	6200	24×10^7
	230,000	<10	<10	<10	<10
No. 7	0	94×10^5	37×10^7	11×10^8	18×10^8
	140,000	29×10^4	19×10^5	72×10^8	26×10^7
2° C.	230,000	11×10^4	66×10^4	13×10^6	19×10^7

these samples had reached 10^6 organisms per gram. The franks in experiment No. 2 had a fairly low initial population, but 460,000 rads only approximately doubled their shelf-life at 10° C.

Microbial flora of irradiated frankfurters. Five representative colonies were picked from the plates for each sample in the experiments discussed in the preceding section. These organisms were then generally identified as to genus. Typical results from the same four experiments listed in Table 3 are summarized in Table 4. The flora of the unirradiated samples were similar to those shown in Table 1. The irradiated franks showed a predominance of yeast and sporeforming bacilli. Other levels of irradiation and the other experiments also gave similar results. Thus, the selective action of these moderate levels of irradiation on the microbial flora coincides with the information presented in Tables 1 and 2 concerning the initial flora and their relative radiation resistance.

SUMMARY

The microbial flora of the surface slime of refrigerated frankfurters has been shown to consist primarily of lactic acid bacteria, yeasts, and micrococci. The most common bacteria were members of the species *Microbacterium thermosphactum,* followed by lactobacilli, leuconostocs, micrococci, and streptococci. Small numbers of gram-negative rods occasionally were found. The radiation resistance of these organisms was determined, and was found to range from about 40,000 rads to 250,000 rads to destroy 99% of the organisms in a broth suspension. The most resistant strains included some yeasts, some streptococci, tetracocci, and homofermentative lactobacilli.

Irradiation of commercially produced frankfurters indicated that 140,000 rads reduced the initial microbial population about 99%, but even this dosage adversely affected the appearance of the product, and did not markedly delay microbial spoilage at 10° C. Irradiation with 460,000 rads approximately

TABLE 4

Microbial flora of irradiated frankfurters

Experiment No. and storage temp.	Irradiation (rads)	Microbial flora[1]	
		Cellophane packed	Vacuum packed
No. 2 10° C.	0	2 Lactobacilli 1 Bacillus 1 Yeast 1 Microbacterium	2 Leuconostocs 2 Micrococci 1 Microbacterium
	230,000	4 Bacilli 1 Yeast	4 Bacilli 1 Yeast
	460,000	5 Bacilli	2 Bacilli 2 Yeasts 1 Tetracoccus
No. 3 10° C.	0	2 Lactobacilli 1 Micrococcus 1 Streptococcus 1 Yeast	3 Yeasts 1 Leuconostoc 1 Tetracoccus
	230,000	5 Yeasts	3 Yeasts 2 Leuconostocs
No. 6 2° C.	0	3 Yeasts 2 Leuconostocs	4 Leuconostocs 1 Yeast
	230,000	5 Yeasts	5 Yeasts
No. 7 2° C.	0	5 Microbacteria	2 Microbacteria 2 Leuconostocs 1 Bacillus
	230,000	5 Bacilli	3 Streptococci 2 Microbacteria

[1] Identity of five isolates.

doubled the time before microbial spoilage at 10° C. However, if the initial microbial population was very low, a dosage of 230,000 rads could greatly delay microbial spoilage at 2° C. The flora developing on the irradiated frankfurters were predominantly yeasts and members of the genus *Bacillus*.

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